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PATENT
16528J-1-8 (formerly 11509-57-5)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:		
Stephen P.A. Fodor et al.	Examiner:	L. Green
Serial No. 07/954,646	Art Unit:	1802
Filed: September 30, 1992		
For: VERY LARGE SCALE IMMOBILIZED POLYMER SYNTHESIS		

COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

DECLARATION OF STEPHEN P.A. FODOR UNDER 37 C.F.R. 1.132

Sir:

I declare and state as follows:

1. I am the Scientific Director and Chief Technical Officer of Affymetrix, Inc. I am an inventor named in the captioned application and am familiar with the material discussed in the specification of the subject application. The specification is the same as the specification which has issued to U.S. Patent No. 5,143,854 ('854). I make reference to the columns and line numbers of this patent below.

2. I and those under my direction and control have conducted experiments using the techniques described in the specification of the captioned application and the '854 patent to synthesize arrays of oligonucleotides and other materials. The techniques described in the application could be used and have been used to synthesize high density arrays of oligonucleotides and other materials.

3. In one particular experiment, we synthesized four arrays of oligonucleotides on a glass support. The support was a 7.62 x 7.62-cm glass substrate. Four arrays, each having sides of approximately 2.56 x 2.56 cm were synthesized

simultaneously on the slide. Each of the four arrays included 4^{10} (1,048,576) different groups of oligonucleotide molecules at known locations on a substrate, i.e., each of the 10-mer oligonucleotides. Each oligonucleotide was formed in a region of about 25 μm on a side.

4. The experiment was conducted by cleaning and silinating the glass substrate. All reactions were conducted at substantially ambient temperature. See column 11, line 51 et al. and col. 17, line 45, of the '854 patent. The surface of the slide was treated to provide polyethylene glycol linker groups on the surface of the substrate, as per col. 12, line 8, of '854. The PEG linker groups were protected with an ortho-nitrobenzyl group (MeNPOC) as per column 13 of '854.

5. A lithographic lamp was used to illuminate the substrate through an in-contact semiconductor mask. See Fig. 8. The mask provided for stripe-shaped reticles. The stripes were moved across and translated relative to the substrate for successive illuminations for each coupling step. See Table 4 of '854. As per col. 14, line 60, in this particular experiment, masking was done with the substrate not in contact with a wash solution.

6. After each masking step, the slide was exposed to an appropriate ortho-nitrobenzyl protected monomer in an acetonitrile solution, with a concentration of 50 mM, in a flow cell. See Fig. 8a of '854. In this particular experiment the coupling sequence was AGCT.... Ten cycles (40 coupling steps) were performed. In each cycle, each base was added once. Accordingly, the masking and monomer addition steps provided for the synthesis of four copies of all 4^{10} 10-mer oligonucleotides on the surface of the substrate. After the 40 coupling steps, the oligonucleotides were deprotected with ammonium hydroxide.

7. To demonstrate that the expected molecules were synthesized, at the known locations, the array was exposed to a 16-mer oligonucleotide target. This target had the sequence 5'-AGTTGTAGTGGATGGT, and is part of exon 7 of the p53 tumor suppression gene. The target was labeled with a fluorescein marker, and exposed to the substrate in a buffered aqueous solution. The hybridized substrate was then examined in a fluorescence scanner such as shown in Fig. 9 of '854.

8. The most strongly bound sequences are identified in Attachment 1. These sequences each correspond to expected 10-mer complements in the p53 tumor suppression gene.

The 7 cells predicted to contain perfect complement oligonucleotides to the target fragment were in the top 50 cells by intensity. Of the 43 remaining cells in the top 50, 3 cells were lit due to a dust particle (clearly visible in the image by its irregular edges), and the remaining 40 are cells containing oligonucleotide probes expected to hybridize well, having at least 8 continuous bases complementary to the target fragment.

Key to results:

Perfect # - perfect match to the #th 10-mer in the target fragment

Mism # - mismatch to the #th 10-mer in the target fragment with at least 8 continuous bases complementary

Perfect +1 - perfect match to last 9 bases of target fragment plus one additional base

Perfect +2 - perfect match to last 8 bases of target fragment

Perfect -1 - perfect match to first 9 bases of target fragment with one additional base

Anom 1 - cells with high intensity

All probe sequences are listed from 3' to 5'. Terminal P is a termination character indicating the end of the probe. Accordingly, it is seen that target bound at the expected locations of the synthesized probes.

9. I further declare that all statements made herein of my knowledge are true and that all statements made on information or belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that willful false statements may jeopardize the validity of the application or any issuing thereon.

Date: 13 Oct 94

By: Stephen P.A. Fodor

Enclosures:
Attachment 1

SPAF:dc
WORK/16528J/000180.P05